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Associations of PrP genotype with lamb production traits in four commercial breeds of British upland and crossing sheep

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ABSTRACT

Selection of sheep on PrP genotype to reduce risks of classical scrapie outbreaks is now widespread in the British sheep industry. However a recurring concern from breeders is that PrP genotype may be unfavourably associated with lamb performance. In this study we report the results from our investigations into this claim using performance data from 12,673 PrP genotyped lambs in four breeds of upland and crossing sheep: Beulah (4014), Blue Faced Leicester (725), Lleyn (5208) and North Country Cheviot (Park) (2726). We also included in the analyses performance data from around 19,000 ungenotyped lambs from the same flocks. The data were supplied by 36 breeders and comprised weights at birth, eight and twenty weeks, and ultrasonic measurements for muscle and fat depth, and deep pedigrees for all animals. Animal (direct) genetic effects and up to three maternal effects were fitted in linear mixed models for each trait. Potential associations with the PrP gene were assessed by fitting either PrP genotype or number of copies of individual alleles carried, as fixed effects. Significant associations between weight traits and PrP genotype were seen only in the North Country Cheviot (Park) breed, and were attributable to the number of copies of VRQ alleles carried. Although there was evidence of significant associations between the number of copies of individual alleles carried and weight traits at different ages in the other three breeds, these associations varied from breed to breed and were not consistent in either their direction, their magnitude or the allele concerned, and none survived Bonferroni corrections for multiple testing. The Blue Faced Leicester breed showed significant associations with PrP genotype and ultrasonic muscle depth, even after Bonferroni correction, due to the number of ARQ alleles carried. In the other breeds, associations between the number of allele copies carried and ultrasonic muscle depth were generally weak and inconsistent. No significant associations were seen for fat depth. In conclusion, few significant associations were seen. Selection on PrP genotype is unlikely to have any noticeable impact on the measured traits in these breeds.

1. Introduction

Concerns over the risk to human health from contaminated food products following the British Bovine Spongiform Encephalopathy outbreak in the 1990s (Ironside et al., 1996, Bruce et al., 1997), led to the launch in 2001 of the Ram Genotyping Scheme (RGS), a part of the National Scrapie Plan (NSP) for Great Britain to combat classical scrapie outbreaks in sheep. The RGS was a selective breeding programme based on the known differing susceptibilities to scrapie infection of the five commonly observed alleles of the PrP gene, viz. ARR, ARQ, AHQ, ARH, VRQ, in which animals with two copies of the VRQ allele are most susceptible to the classical form of the disease, while those with two copies of the ARR allele are the most resistant (Hunter et al., 1994; Baylis and Goldmann, 2004). The RGS aimed to reduce and eventually eliminate susceptible genotypes from the national pedigree flock by selecting on these alleles only as there was no EU policy for selection at other codons.

At the advent of the NSP, various anecdotal claims were made, based on visual observations of difficult-to-measurable characteristics, that many animals with superior PrP genotypes

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displayed less than desirable phenotypes. In response, a research project was initiated in 2005 to examine potential associations of PrP genotype with production traits, using objectively measured performance data collected from twelve commercial British sheep breeds, along with three further research flocks. Results for the research flocks have been reported elsewhere, (Man et al., 2006; Sawalha et al., 2007), and we have previously reported our results for the hill and lowland sectors of the industry (Moore et al., 2009a and Moore et al., 2009b). Earlier studies on performance traits in important European sheep breeds that ultimately effected EU policy decisions were summarised by Sweeney and Hanrahan, 2008. However, these studies had mostly involved non-British breeds or referred to traits of little importance to the British sheep industry, e.g., milk production traits (Alvarez et al., 2006). Other studies covered by the review had used limited datasets (de Vries et al., 2006; Vitezica et al., 2006) or datasets from experimental rather than commercial flocks (Man et al., 2006; Sawalha et al., 2007). Only in the recent study of Pritchard et al., 2008, has commercial lamb performance data been examined in an important and numerically large British breed, the Welsh Mountain.

Upland and crossing sector breeds are a remaining gap in our knowledge of possible associations between PrP genotype and lamb performance traits for British sheep breeds. Individually the four breeds considered in this paper are important to the British industry. The Beulah is a regional breed, not normally found outside Wales and the neighbouring counties, however present in large enough numbers to be considered of national importance, contributing 3.2% (4th highest) of the national flock with some 571,000 ewes mated in 2003, of which 498,000 were pure bred (Pollott et al., 2006). The North Country Cheviot is found locally in the northern and border counties of Scotland, with smaller numbers in Wales. According to Pollott et al., 2006, the North Country Cheviot is the 5th largest pure breed comprising 2.87% the national flock; however the authors (Pollott et al.) do not distinguish between the Hill and Park types of North Country Cheviot in their research. The Lleyn has steadily grown in popularity in Britain and while it was initially classified as a lowland breed, it is now considered to be a versatile breed and is found in many upland situations, especially on farms that have restocked since the Foot and Mouth Disease outbreak in 2001 (Lleyn Breed Society, 2009). In 2003 it was the 8th most popular breed comprising 1.56% the national flock (Pollott et al., 2006). The Blue Faced Leicester breed is an important crossing breed; in 2003 around 30,000 Blue Faced Leicester rams were crossed with 1.2 million Swaledale and Scottish Blackface ewes, producing North Country and Scotch Mules, which together comprise 16.4% the national flock (Pollott et al., 2006). Consequently, this paper presents an analysis of lamb production traits and their association with PrP genotype in four upland and crossing sheep breeds.

2. Materials and methods

2.1. Datasets and traits

A total of 32,196 lamb performance and pedigree records from four British upland and crossing breeds were supplied by the Meat and Livestock Commission (MLC). These data were obtained from 8 Beulah, 6 Blue Faced Leicester, 9 Lleyn and 13 North Country Cheviot (Park) commercial pedigree breeders who had responded positively to the invitation to participate in the research. Unique individual identifications were available for all animals and these were used to match the PrP genotypes supplied by the National Scrapie Plan Action Centre (NSPAC) to their pedigree performance data. PrP genotypes were not available for all animals; but performance data from all animals born in each flock were included in the datasets (as described below), providing a complete dataset from each flock from 1997 to 2005 (2006 for Beulah), containing a total of 12,673 lambs with PrP genotype information. The number of recorded lambs present differed per breed, as did the proportion of lamb records (19.7–43.8%) that could be matched with PrP genotypes (Table 1). Birth weight data were supplied only by a subset of breeders and the birth weight models were adjusted to reflect this.

Six traits were analysed, and summary statistics for each trait are shown by breed in Table 2. The traits were defined as follows, *Birthweight:* weight (kg) at or within 24 h of birth; *Eight-week weight:* actual lamb weight (kg); *Scan weight:* actual lamb weight (kg) at ultrasonic scanning (approximately 20 weeks); *Average Daily Weight Gain:* weight gain (kg/day) between eight-week weight and scanning weight divided by the interval in days between weighing and scanning; *Ultrasonic Muscle Depth:* muscle depth (mm) at the level of the third lumbar vertebra; *Ultrasonic fat depth:* fat depth (mm) at the level of the third lumbar vertebra. Fat depth data were right skewed from the normal distribution curve and so were square root transformed prior to analysis.

2.2. PrP genotypes

NSPAC supplied PrP genotypes that had been obtained using a variety of proprietary commercial genotyping methodologies, chiefly SNP assays. In all cases the ovine PrP gene was genotyped at polymorphic codons 136(A/V), 154 (R/H) and 171(Q/R/H) to discriminate between the five major alleles: ARR, ARQ, ARH, AHQ and VRQ. PrP genotypes were merged with performance datasets and cleaned using SAS software Version 9.1 prior to analysis.

Table 1
Dataset structure by breed.

Breed	No. of breeders	No. of lambs	No. of genotyped lambs	Percentage of lambs genotyped	Year range
Beulah	8	10,115	4014	39.7	1997–2006
Blue Faced Leicester	6	3689	725	19.7	1997–2005
Lleyn	9	12,176	5208	42.8	1997–2005
North Country Cheviot (Park)	13	6216	2726	43.8	1999–2005

Table 2
Means by trait and breed.

Trait [a]	Breed [b]	Mean	*n*	CV%	Min	Max	No. of sires
BWT (kg)	BEU	3.91	2564	19.0	1.4	7.0	78
	LL	4.04	10,176	24.0	0.5	8.4	138
	BFL	4.91	1982	23.5	1.3	9.1	76
	NCCP	4.87	2821	27.8	1.0	10.0	79
W8W (kg)	BEU	19.4	10,115	24.5	4.0	44.5	182
	LL	22.5	12,175	24.2	5.0	46.0	153
	BFL	25.6	3563	25.1	7.0	48.0	125
	NCCP	27.1	5080	30.6	4.0	57.0	129
SWT (kg)	BEU	30.3	9086	20.9	11.0	74.0	182
	LL	32.8	3281	20.1	13.5	56.5	88
	BFL	44.1	3175	19.5	17.0	74.0	121
	NCCP	41.1	4038	21.0	16.0	80.0	125
UMD (mm)	BEU	21.4	8034	14.5	9.0	33.0	180
	LL	23.2	2426	15.5	10.0	34.0	89
	BFL	22.7	3141	14.9	10.0	35.0	120
	NCCP	24.2	3829	13.0	14.0	36.0	121
UFD ($mm^{0.5}$) [c]	BEU	1.48	8035	25.2	0.17	3.28	180
	LL	1.53	2349	20.8	0.57	2.93	89
	BFL	1.57	3140	28.0	0.33	3.37	120
	NCCP	1.47	3829	25.3	0.36	3.16	121
ADWG (kg/day)	BEU	0.133	9078	38.7	0.071	0.484	182
	LL	0.126	3280	38.5	0.140	0.393	89
	BFL	0.249	3174	28.4	0.024	0.525	120
	NCCP	0.187	4038	37.9	0.115	0.667	125

[a] BWT: birth weight; W8W: eight-week weight; SWT: scan weight; UMD: ultrasonic muscle depth; UFD: ultrasonic fat depth; ADWG: average daily weight gain from 8–20 weeks; *n*, number of observations; CV, coefficient of variation; Min, minimum; Max, maximum.
[b] BEU: Beulah; BFL: Blue Faced Leicester; LL: Lleyn; NCCP: North Country Cheviot (Park).
[c] Square root transformed.

2.3. Statistical methodology

Linear mixed models were used for all traits and included direct (animal) genetic effects and up to three maternal effects: maternal genetic, permanent environmental (PE) and temporary (litter) effect. All models were of the form:

$$\mathbf{y} = X\mathbf{b} + Z_1 a + Z_2 m + Z_3 l + Z_4 pe + e$$

Where **y** is the vector of observations for each trait, **b** the vector of fixed effects, *a* the additive direct (animal) genetic effects, *m* the additive maternal genetic effects, *l* the maternal temporary environmental effects, *pe* the permanent environmental effects, and *e* the random residual effects. X, Z_1, Z_2, Z_3, Z_4 are design matrices relating fixed and random effects to observations. All models included fixed effects of flock/breeder, year, sex, maternal age, litter size and type of rearing, and covariates of birth date and age at which measurements were obtained, i.e., age at weighing and age at scanning. Effects contributing significantly to the global model fit, as assessed by the likelihood ratio test, were included in the final model. In all cases the most economical random effects model was selected and analysed using ASReml v 2.0 (Gilmour et al., 2007). This resulted in slightly different models being fitted for the same trait in different breeds; however, the primary aim was to define the best fitting model for the purpose of assessing PrP effects, rather than to estimate and compare variance components.

2.4. Testing for associations with PrP genotype

Potential associations between each performance trait and the PrP gene were estimated from the PrP fixed effect, with ungenotyped animals fitted as an additional category. Depending on the breed involved, between six and nine PrP genotypes were included, e.g. ARR/ARR, ARR/ARQ, ARR/AHQ, etc. Additionally, animals were then classified according to the number of copies of each of the PrP alleles carried (i.e. homozygous, heterozygous, or allele not present) and were examined in turn, e.g. ARR/ARR, ARR/xxx and xxx/xxx, where xxx represents non-ARR alleles, etc., again fitting ungenotyped animals as an additional category. As only a proportion of the animals in each dataset were genotyped, the significance of the PrP genotype or allele effects was assessed after fitting a fixed effect of "genotyped or ungenotyped". For most traits, genotyped lambs had superior trait values to ungenotyped lambs, possibly reflecting preferential genotyping by breeders of better performing lambs.

Exploration of PrP associations in these analyses involve a large number of independent comparisons, i.e. four breeds and several traits, hence there is an increased risk of obtaining apparently significant results by chance alone. A conservative means of adjusting for this is the Bonferroni correction in which the significance level (a) is adjusted to $[1-(1-a)^{1/n}]$,

Table 3
PrP allele frequencies (%) by breed.

Breed	ARR	ARQ	AHQ	ARH	VRQ
BEU	69.4	26.6	2.4	0.0	1.6
BFL	69.2	15.9	14.9	0.0	0.0
LL	74.4	10.9	7.0	7.2	0.5
NCCP	58.9	30.4	9.0	0.2	1.5

BEU: Beulah; BFL: Blue Faced Leicester; LL: Lleyn; NCCP: North Country Cheviot (Park).

Table 4
Estimates for direct heritability, temporary dam (litter effect), permanent environmental effect of dam and maternal heritability for Beulah, Blue Faced Leicester, Lleyn and North Country Cheviot (Park) breeds.[a]

Parameter[a]	Breed[b]	BWT[b]	(SE)	W8W	(SE)	SWT	(SE)	ADWG	(SE)	UMD	(SE)	UMD SWT	(SE)	UFD	(SE)	UFD SWT	(SE)
h^2_d	BEU	0.05	(0.03)	0.26	(0.03)	0.29	(0.03)	0.24	(0.02)	0.31	(0.03)	0.30	(0.03)	0.37	(0.03)	0.39	0.03
	BFL	0.13	(0.05)	0.11	(0.04)	0.23	(0.05)	0.35	(0.05)	0.17	(0.04)	–	–	0.23	(0.04)		
	LL	0.25	(0.03)	0.25	(0.02)	0.40	(0.05)	0.24	(0.05)	0.10	(0.05)	–	–	0.38	(0.06)	–	–
	NCCP	0.31	(0.05)	0.18	(0.03)	0.30	(0.04)	0.22	(0.03)	0.34	(0.04)	–	–	0.42	(0.04)		
LITT^2	BEU	0.29	(0.03)	0.31	(0.02)	0.21	(0.02)	0.21	(0.02)	0.21	(0.02)	0.19	(0.02)	0.21	(0.02)	0.14	(0.02)
	BFL	0.45	(0.01)	0.17	(0.03)	0.20	(0.03)	0.19	(0.02)	–	–	–	–	0.22	(0.03)		
	LL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	NCCP	0.48	(0.01)	0.30	(0.02)	0.32	(0.03)	0.26	(0.03)	0.20	(0.03)	–	–	0.25	(0.03)	–	–
PE^2	BEU	0.09	(0.01)	0.05	(0.02)	–	–	–	–	–	–	–	–	–	–	–	–
	BFL	0.07	(0.04)	–	–	–	–	–	–	0.19	(0.03)	–	–	–	–	–	–
	LL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	NCCP	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
h^2_m	BEU	0.16	(0.04)	0.08	(0.02)	0.11	(0.01)	0.05	(0.01)	0.04	(0.01)	0.03	(0.01)	0.08	(0.01)	0.05	(0.01)
	BFL	0.06	(0.05)	0.06	(0.02)	0.10	(0.02)	0.12	(0.02)	0.04	(0.02)			0.05	(0.02)	–	–
	LL	0.14	(0.01)	0.14	(0.01)	0.12	(0.03)	0.02	(0.02)	0.06	(0.03)	–	–	0.05	(0.02)	–	–
	NCCP	0.10	(0.03)	0.07	(0.02)	0.04	(0.02)	–	–	0.03	(0.02)	–	–	–	–	–	–

[a] h^2_d: direct heritability (σ^2_a/σ^2_p); LITT^2: σ^2_l/σ^2_p; PE^2: $\sigma^2_{perm}/\sigma^2_p$; h^2_m: maternal heritability (σ^2_m/σ^2_p); where σ^2_a, σ^2_m, σ^2_l, σ^2_{perm} and σ^2_p are the additive genetic, maternal genetic, litter, permanent environment and phenotypic variance, respectively.
[b] See Table 2 for breed and trait descriptions.

where there are n independent comparisons (hypotheses) being made. Correcting for four independent datasets, the 0.05 significance threshold becomes 0.0127, for four datasets and two independent traits (body size and carcass composition) the threshold is 0.0064, and for four datasets and three traits (body size, fat and muscle depth) the threshold is 0.0043.

3. Results

3.1. PrP allele distribution

There were substantial differences in PrP allele frequencies among the four breed datasets, as shown in Table 3. All five common PrP alleles were present in the Lleyn and North Country Cheviot (Park) datasets; however the Beulah and Blue Faced Leicester datasets lacked the ARH allele. The most frequent allele in all breeds was ARR, and ARQ and AHQ alleles were present in all breeds but at lower frequencies. Only the North Country Cheviot (Park) and Lleyn datasets carried the ARH allele, with the relatively high frequency in the Lleyn breed (7%) possibly reflecting introduction of animals from the Texel breed, where this allele is common. There were no VRQ alleles present in the Blue Faced Leicester, and the other three breeds showed low VRQ frequencies. In general, allele frequencies for these four breeds are similar to those presented by Eglin et al. (2005). Based on allele frequencies shown in Table 3, observed genotype frequencies were close to expectation (results not shown) with only the Lleyn breed differing from Hardy–Weinberg expectations ($p<0.01$). In this case there was a relative excess of ARR/VRQ, compared to other VRQ carrying genotypes and an excess of ARQ/ARQ genotypes. Plausible explanations include non-random, particularly as animals carrying ARR/VRQ genotypes were permitted under NSP regulations, and population substructure due to the historical introduction of Texel animals into the breed.

3.2. Genetic and environmental parameters

Estimates of genetic parameters for all breeds and traits (Table 4) were in overall agreement with previous published studies in other breeds Where significant, the litter (LITT^2) effect was moderate and ranged from 0.17 to 0.48, similar to the range apparent in hill and lowland breeds (Moore et al., 2009a; unpublished results). The permanent environmental effect of the dam (PE^2) was fitted in all cases however was seldom significant. Overall, maternal genetic effects (h^2_m) were low and generally declined thereafter in traits more distant from birth. A similar pattern was evident in the maternal genetic effects of the three hill breeds North Country Cheviot (Hill), Scottish Blackface and Welsh Mountain (Moore et al., 2009a) and also the three lowland breeds Charollais, Poll Dorset and Texel studied in this project (unpublished results).

3.3. PrP associations with performance traits

3.3.1. Live weights and weight gain

Only in the North Country Cheviot (Park) breed was a significant association found between PrP genotype and weight at birth ($p<0.05$), with lambs carrying one copy of the VRQ allele being 0.34 kg heavier than lambs with no copies of the VRQ allele ($p=0.02$, Fig. 1).

No significant associations were detected between eight-week weight and PrP genotype in any of the four breeds, although analyses of PrP alleles did result in some statistically significant associations. In the Beulah breed, significant associations were seen with the number of ARR and ARQ alleles carried. Lambs with two copies of the ARR allele were 0.13 kg heavier than lambs with zero copies ($p=0.05$, Table 5, Fig. 2), and lambs with two copies of the ARQ allele were 0.41 kg lighter than lambs with zero copies ($p=0.02$) at eight-week weights (Table 5). Blue Faced Leicester lambs carrying two copies of the ARQ allele were 2.67 kg lighter than those with only one copy ($p=0.03$), although these

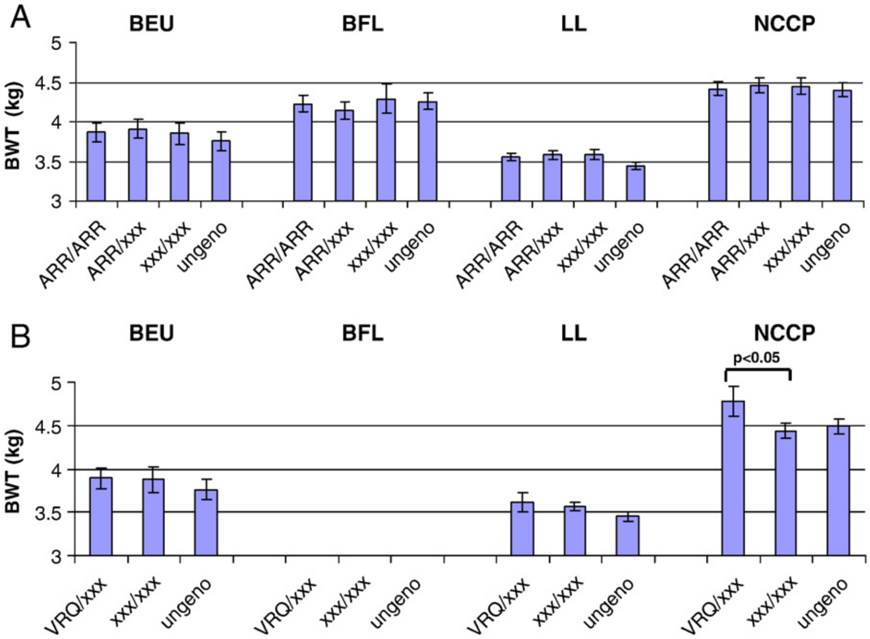

Fig. 1. Least squares means (and standard errors) for birth weight (BWT) of animals classified by the number of copies of (A) ARR and (B) VRQ alleles, where BEU is Beulah, BFL is Blue Faced Leicester, LL is Lleyn and NCCP is North Country Cheviot (Park). Significant genotype contrasts and their p value are indicated on the figure.

lambs comprised only 2.1% of the genotyped lambs for this breed. No associations were seen in either of the other two breeds with number of copies of PrP alleles carried.

No significant associations were detected between scan weight and PrP genotype in any of the four breeds studied; however, significant associations were seen with number of copies of allele carried in the Beulah and Lleyn breeds. In the Beulah breed, lambs with one copy of the ARR allele were 0.43 kg lighter ($p = 0.04$) at scanning weight than those with no copy (Table 5, Fig. 3). In the Lleyn breed, lambs with two copies of the AHQ allele were significantly heavier by 3.5 kg ($p = 0.05$) than those with only one or zero copies; however only nine lambs were present in this category.

Analysis of data for all four breeds failed to detect significant associations of PrP genotype with average daily weight gain (Table 5, Fig. 4). However, the number of ARR and ARQ allele copies carried had significant effects on the average daily weight gain in Beulah lambs (Table 5). Lambs from this breed with one copy of the ARR allele had a 0.01 kg per day lower weight gain than those with two copies ($p = 0.02$), while lambs with two copies of the ARQ allele gained an average 0.008 kg per day more than lambs with no copies of this allele ($p = 0.05$).

In summary, some apparently significant associations have been observed between PrP genotype or alleles and live weight traits. However, when Bonferroni corrections are performed to correct for the fact that multiple comparisons have been made, none of the associations remain significant.

3.4. Muscle and fat depth

There were significant associations between muscle depth and PrP genotype in the Blue Faced Leicester breed only; however nominally significant associations with number of allele copies carried were seen in all four breeds analysed (Table 5). In the Beulah breed, lambs with two copies of the ARR allele had 0.19 mm greater muscle depth than lambs with one or zero copies ($p = 0.05$, Fig. 5). A significant

Table 5
Tests of significance (P values) for different traits and analyses differing in genotypic classifications for the four breeds.

Classification	Breed [a]	BWT [b]	W8W	SWT	ADWG	UMD	UFD
PrP genotype	BEU	0.81	0.17	0.15	0.07	0.25	0.67
	BFL	0.47	0.07	0.36	0.71	**0.05**	0.36
	LL	0.84	0.62	0.25	0.66	0.54	0.92
	NCCP	**0.05**	0.78	0.54	0.21	0.16	0.45
ARR carriers	BEU	0.40	**0.05**	**0.04**	**0.02**	**0.05**	0.19
	BFL	0.30	0.51	0.91	0.42	0.41	0.61
	LL	0.66	0.23	0.95	0.99	0.41	0.28
	NCCP	0.70	0.57	0.34	0.08	**0.04**	0.54
ARQ carriers	BEU	0.34	**0.02**	0.12	**0.05**	0.08	0.17
	BFL	0.98	**0.03**	0.16	0.89	**0.01**	0.37
	LL	0.71	0.46	0.37	0.85	0.49	0.45
	NCCP	0.31	0.54	0.14	0.08	0.36	0.36
ARH carriers	BEU [b]						
	BFL [b]						
	LL	0.11	0.18	0.38	0.21	0.71	0.89
	NCCP [b]						
AHQ carriers	BEU	0.45	0.92	0.51	0.51	0.99	0.40
	BFL	0.47	0.58	0.81	0.68	0.59	0.20
	LL	0.90	0.26	**0.05**	0.33	0.60	0.80
	NCCP	0.08	0.36	0.48	0.89	0.92	0.57
VRQ carriers	BEU	0.92	0.34	0.26	0.29	0.46	0.37
	BFL [b]						
	LL	0.73	0.86	0.89	0.73	**0.05**	0.73
	NCCP	**0.02**	0.92	0.92	0.30	0.44	0.15

Figures in bold represent significant results.
[a] See Table 2 for breed and trait descriptions.
[b] Alleles not present in this breed.

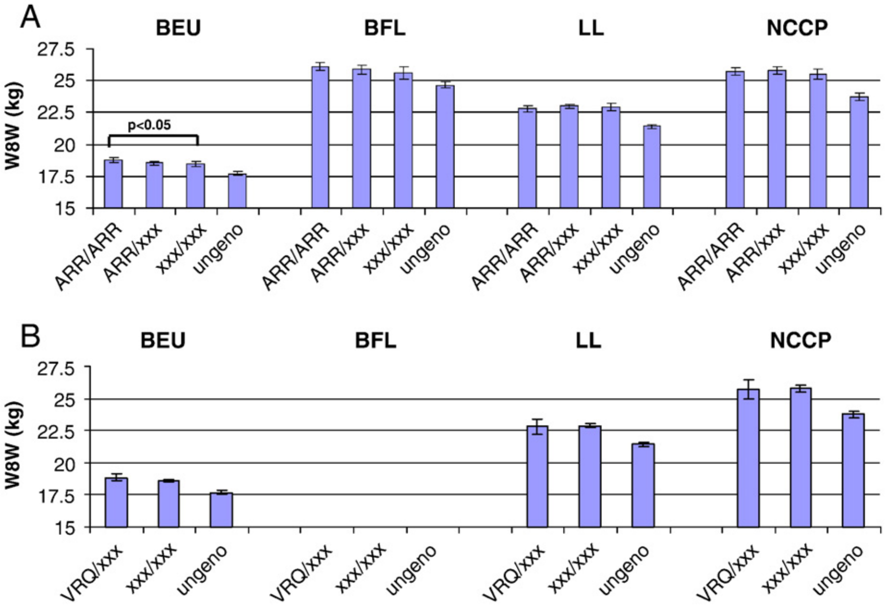

Fig. 2. Least squares means (and standard errors) for eight-week weight (W8W) of animals classified by the number of copies of (A) ARR and (B) VRQ alleles, where BEU is Beulah, BFL is Blue Faced Leicester, LL is Lleyn and NCCP is North Country Cheviot (Park). Significant genotype contrasts and their p value are indicated on the figure.

association ($p = 0.05$) of PrP genotype with ultrasonic muscle depth was seen in ARQ/ARQ lambs from the Blue Faced Leicester breed, and a significant association ($p = 0.01$) was also seen for lambs carrying two copies of the ARQ allele. ARQ/ARQ lambs had 1.5 mm lower muscle depth than lambs with one copy of this allele and 1.2 mm lower muscle depth than lambs carrying zero copies. Lleyn lambs showed significant associations with the VRQ allele where lambs with one copy had 2.5 mm greater muscle depth than lambs with zero copies ($p = 0.05$, Fig. 5). The North Country Cheviot (Park) lambs with one copy of the ARR allele had 0.4 mm greater muscle depth than lambs with no copies of this allele

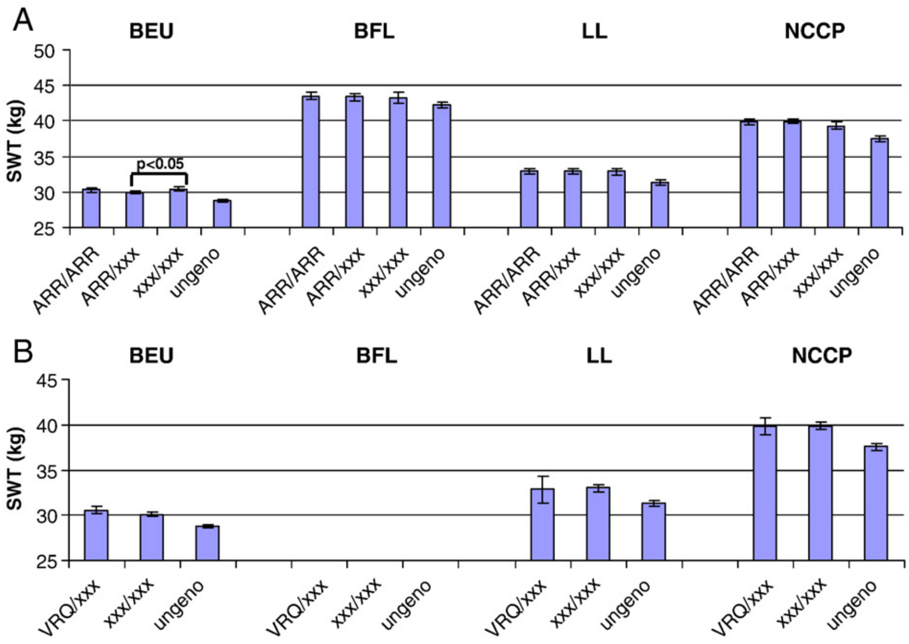

Fig. 3. Least squares means (and standard errors) for scan weight (SWT) of animals classified by the number of copies of (A) ARR and (B) VRQ alleles, where BEU is Beulah, BFL is Blue Faced Leicester, LL is Lleyn and NCCP is North Country Cheviot (Park). Significant genotype contrasts and their p value are indicated on the figure.

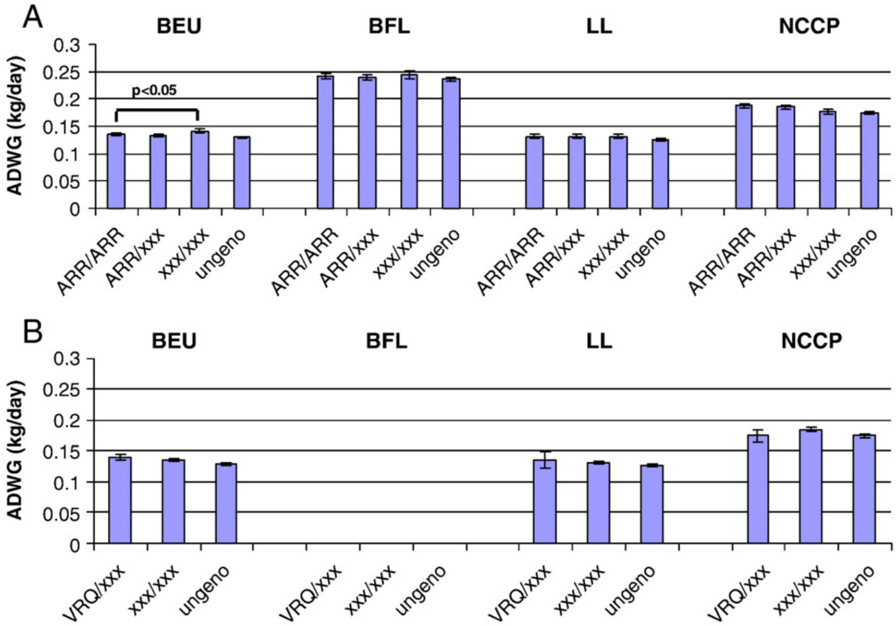

Fig. 4. Least squares means (and standard errors) for average daily weight gain (ADWG) of animals classified by the number of copies of (A) ARR and (B) VRQ alleles, where BEU is Beulah, BFL is Blue Faced Leicester, LL is Lleyn and NCCP is North Country Cheviot (Park). Significant genotype contrasts and their *p* value are indicated on the figure.

($p = 0.04$, Fig. 5). Of these associations, only the association seen in the Blue Faced Leicester dataset remained significant after Bonferroni correction for multiple testing. No significant associations were found between fat depth and PrP genotype or number of copies of allele carried in any of the breeds studied (Table 5, Fig. 6).

4. Discussion

A number of apparently significant associations between PrP genotype and lamb performance traits were detected in all four of the breeds included in this study; however, these tended to be weak and inconsistent, with only one remaining

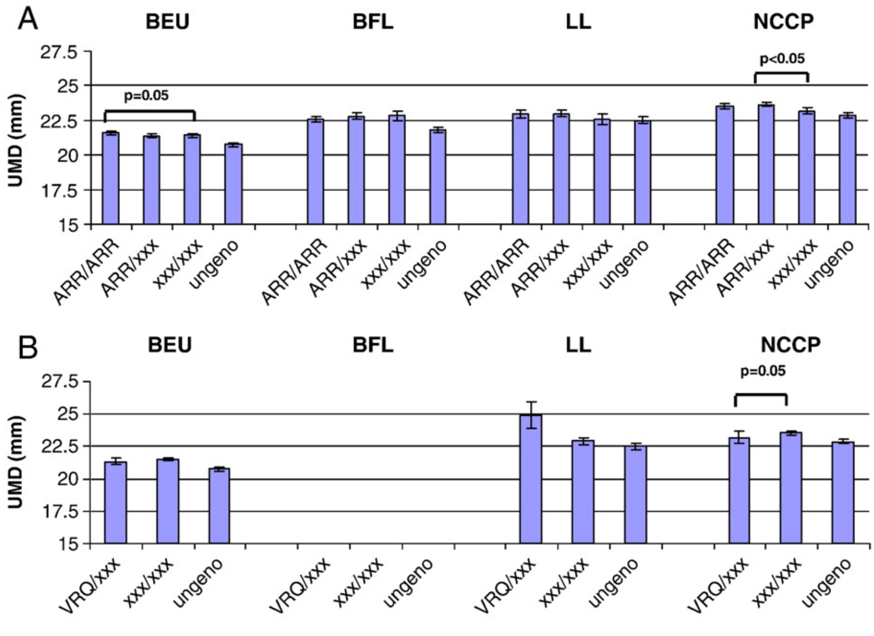

Fig. 5. Least squares means (and standard errors) for ultrasonic muscle depth (UMD) of animals classified by the number of copies of (A) ARR and (B) VRQ alleles, where BEU is Beulah, BFL is Blue Faced Leicester, LL is Lleyn and NCCP is North Country Cheviot (Park). Significant genotype contrasts and their *p* value are indicated on the figure.

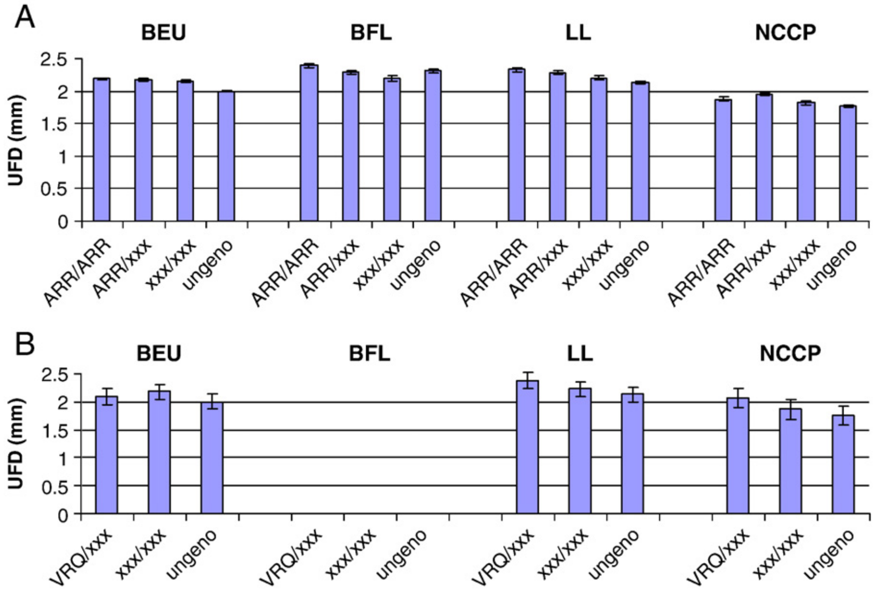

Fig. 6. Least squares means for backtransformed ultrasonic fat depth (UFD) of animals classified by the number of copies of (A) ARR and (B) VRQ alleles, where BEU is Beulah, BFL is Blue Faced Leicester, LL is Lleyn and NCCP is North Country Cheviot (Park). Significant genotype contrasts and their *p* value are indicated on the figure. Standard errors are not shown for backtransformed data.

significant following Bonferroni correction. Whilst it is likely that most of the apparently significant results are false positives, it is nevertheless useful to consider their possible implications.

In the Beulah breed, lambs with one copy of the ARR allele had a slight (although not significant) advantage at eight-week weight over those with two or no copies of this allele. However, they had significantly lower average daily weight gains, and as a result they were significantly lighter at scan weight. In addition, these lambs also had significantly less muscle depth than lambs with two copies of the ARR allele. Therefore, the evidence suggests that ARR/ARR lambs would reach finishing weights faster and be better conformed than lambs with other genotypes included in this dataset. Both these effects are commercially desirable and indicate that selection of animals towards PrP resistant genotypes may be advantageous in terms of lamb growth rates and therefore profitability.

The Blue Faced Leicester is a highly important breed to the British industry. Around 30,000 Blue Faced Leicester rams are crossed with 1.2 million Swaledale and Scottish Blackface ewes annually, producing North Country and Scotch Mules, which together comprise 16.4% the national flock (Pollott et al., 2006). Around one million male lambs from these crosses are slaughtered while ewe lambs go on for breeding and are crossed with terminal sires; all progeny are normally slaughtered for the meat industry. A PrP genotype association was observed in this breed with muscle depth, being entirely due to the reduced muscle depths seen in lambs with two copies of the ARQ allele. Our data also show that the same lambs were significantly lighter at eight weeks of age. Although not significant, the same lambs remained lighter at scanning and carried less fat than lambs with any other combination of the three alleles found in the Blue Faced Leicester breed. Together, these effects have a large impact on carcase composition for Blue Faced Leicester lambs carrying two copies of the ARQ allele, but only around 2% of the breed is likely to fall into this category, and this proportion may decrease further with selection favouring the ARR allele.

To fully interpret the impact of PrP genotype frequency changes on commercial traits in the Blue Faced Leicester breed, it is useful also to consider the breeds with which they are crossed. Man et al., 2006, did not find any associations of PrP genotype with performance traits in a research flock of Swaledale ewes, while Sawalha et al., 2007, found few associations of PrP genotype with carcase traits in two research flocks of Scottish Blackface sheep. Moore et al., 2009a did detect significant effects in commercial Scottish Blackface lambs, however none had any effect on the eventual carcase conformation of the weaned lamb and, in addition, none remained significant after Bonferroni correction. Because it is the ARQ allele that is seemingly disadvantageous for lamb performance traits in the Blue Faced Leicester breed, with this result withstanding Bonferroni correction, the general decrease in ARQ allele frequency that has most likely occurred as a result of selection on PrP genotype can be viewed as favourable.

Little impact of altering PrP genotype frequencies would be expected for lamb production traits in the Lleyn breed. Although two significant results were found, these were with minority genotypes. Lambs with two copies of the AHQ allele ($n = 9$) were significantly heavier than those with one or no copies, while lambs with one copy of the VRQ allele (again, $n = 9$) had significantly greater muscle depths than lambs with no copies of this allele. There were no lambs from the Lleyn dataset carrying two copies of the VRQ allele. The Texel breed is thought to have played a considerable part in the genetic makeup of the Lleyn breed, as these are the only two British breeds that carry a significant percentage of ARH alleles. Only the Texel breed has shown an association with this allele (Moore et al., 2009b).

The North Country Cheviot (Park) breed showed a significant PrP genotype and VRQ allele association with heavier birth weight. The data also showed that the VRQ carrying lambs (around 3% total genotyped lambs in this breed, $n=24$) were no heavier at weaning than non carriers, indicating that there would be no losses to the breed with regard to carcase composition or performance should the VRQ allele disappear. Heavier birth weights are sometimes associated with dystocia and hence reduced lamb survival; however, Gubbins et al., 2009, showed that there were no survival associations with PrP genotype or number of copies of alleles carried in any of the four breeds involved in this study. The North Country Cheviot (Hill) breed, closely related to the Park breed, also showed significant associations with birth weight, however these were due to a small number of homozygous AHQ lambs (Moore et al., 2009a). ARR-carrying North Country Cheviot (Park) lambs showed significant associations with ultrasonic muscle depths, being higher than those for non-ARR-carrying lambs. This result differs from that seen in the Hill type, where there were no associations seen with this allele for any traits.

The low numbers of VRQ alleles seen in three of the breeds in this study, and indeed the complete absence of VRQ alleles in the Blue Faced Leicester breed, has perhaps affected the ability to detect associations of the VRQ allele with performance traits; however, the actual biological associations of VRQ alleles with performance traits are unlikely to have been affected by prior selection pressure exerted on PrP genotype. Eglin et al., 2005, described other, rarer, breeds of British sheep with high frequencies of VRQ carrying genotypes, e.g. Exmore Horn (12.8%), Devon Closewool (11.2%); such breeds were not included in this study as breeders were unable to provide large enough datasets of performance traits for analysis. The VRQ allele is at a high frequency in the Dorset Horn breed (11.8%, Eglin et al., 2005), however in a previous study (Moore et al., 2009b) we found no associations between lamb performance traits and PrP genotype in this breed at all.

To conclude, results for these four breeds are, on the whole, weak and inconsistent with previous results obtained using commercial data from several hill and lowland breeds (Moore et al., 2009a,b). Nor do they show consistency with parallel studies performed on research flocks (Man et al., 2006; Sawalha et al., 2007; Sweeney and Hanrahan, 2008). The general lack of consistency in the associations of PrP genotype with lamb performance traits in this and in previous studies, along with the lack of evidence found from the recent summary of literature on PrP association studies, leads to the conclusion that any associations with these traits are breed specific and therefore inconsistent within and across sectors of the industry. Therefore, there is no compelling evidence to reject the null hypothesis that there is no general association of PrP genotype with lamb performance and carcase traits.

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